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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Compositions containing cis-isomers of a carotenoid compound

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Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The number of transformed cells was determined by the number of colonies obtained on the selective medium. The results are the mean of three independent experiments. Error bars represent the standard deviation.

Compositions containing cis-isomers of a carotenoid compound

The present invention relates to a primary composition that includes at least one carotenoid-containing material enriched in cis-isomers of the carotenoid compound, and process of forming the same. It also relates to an oral composition that contains the primary composition in a foodstuff, in a food supplement, in a cosmetic preparation or in a pharmaceutical preparation.

Background of the invention

Compositions available on the market that include carotenoids, namely lycopene, are already known. Lycopene is a natural product which is known to have multiple roles, in particular that of an antioxidant that alleviates chronic diseases. Lycopene is present in various natural products, in particular tomatoes, melons, guavas and grapefruit. The composition generally available on the market which comprises lycopene is an oleoresin. The problem with this oleoresin is that the lycopene present therein is insufficiently bioavailable.

For example, European patent 278 284 relates to a pulverulent composition comprising a synthetic carotenoid. The problem with this composition is that it cannot be used in the food field and, moreover, it is envisaged for a coloring purpose.

Although EP 1 289 383 provides a primary composition that includes at least one lipophilic bioactive compound and a whey protein in an amount effective to increase the bioavailability of the lipophilic bioactive compound, there is still a need for a carotenoid-containing product which has higher bioavailability and bioefficacy than the products currently on the market. The carotenoid-containing composition must be soluble in lipids and organic solvents, less prone to crystallization, and have a lower tendency to aggregate.

Summary of the invention

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Accordingly, it is a first object of the invention to provide a primary composition comprising at least one carotenoid-containing material enriched in cis-isomers, the cis isomer content being in an amount effective to increase the bioavailability and/or bioefficacy of the carotenoid compound.

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The carotenoid-containing material is an extract, concentrate or oleoresin advantageously obtained, extracted, enriched or purified from a plant or a vegetable material, microorganism, yeast or product of animal origin.

10

The preferred form of the primary composition is as an additive in a foodstuff for oral administration, such as in a nutritional composition, a food supplement, a pet food product, a cosmetic preparation or in a pharmaceutical preparation.

15

The invention also relates to methods of forming the primary composition, the food supplement, cosmetic preparation or pharmaceutical preparation containing the same.

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The process for the preparation of a primary composition according to the present invention comprises subjecting a carotenoid-containing material as described above under conditions sufficient to increase its content in cis-isomers to an amount effective to increase the bioavailability and/or bioefficacy of the carotenoid compound in the primary composition.

25

In a further aspect, the invention provides the use of a primary composition comprising at least one carotenoid-containing material enriched in cis-isomers, for the preparation of an oral, cosmetic or pharmaceutical composition intended for improving skin health, in particular for photoprotection of the skin or for protecting skin tissue against ageing.

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The invention also relates to the use of said primary composition for the preparation of an oral, cosmetic or pharmaceutical composition for preventing or treating cardiovascular diseases or cancers.

5 The present invention now makes available to the consumer an improved composition obtained from natural products. It provides a primary composition having a cis-isomer content of carotenoid higher than existing compositions. The primary composition provides carotenoids in a particularly highly bioavailable and/or bioeffective form, which is soluble in lipids and organic solvents, less prone to
10 crystallization, and having a lower tendency to aggregate.

The features of the present invention can be best understood together with further objects and advantages by reference to the following description, taken in connection with the accompanying drawings.

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Brief description of the drawings

Fig. 1 shows a typical HPLC chromatogram of lycopene isomers generated by microwave irradiation, illustrating the products obtained by the process according to the
20 present invention.

Fig. 2 outlines the advantage of microwave irradiation versus conventional heating.

Detailed description of the invention

According to the first object, a primary composition comprising a carotenoid-containing material enriched in cis-isomers of said carotenoid compound, is concerned.

30 In a preferred embodiment, the carotenoid-containing material is in the form of an extract, a concentrate or an oleoresin, for example. Within the following description,

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the term "oleoresin" is understood to mean a lipid extract of a carotenoid-containing material, which includes carotenoids, triglycerides, phospholipids, tocopherols, tocotrienols, phytosterols and other less significant compounds.

5 Advantageously, the carotenoid-containing material is an extract, concentrate or oleoresin, which is obtained, extracted, enriched or purified from a plant or vegetable material, a microorganism, a yeast or a product of animal origin. It is further subjected to a treatment to increase its cis-isomer content of carotenoid, as described below.

10 If the source of carotenoid is from plant origin, it may be vegetables, leaves, flowers, fruits and other parts of the plant. In a preferred embodiment, the source of carotenoids is from tomatoes (i.e., whole tomato, tomato extract, tomato flesh, tomato puree, tomato skin, with or without the seeds), carrots, peaches, apricots, oranges, melons, guavas, papayas, grapefruit, rosehips, soya, green tea, green coffee beans,
 15 spices such as ginger or others, grapes and cocoa, for example. Suitable plant or vegetable concentrates are obtainable e.g. by drying or freeze-drying the fresh-cut plants or vegetables or the respective roots, fruits or seeds thereof and then optionally grinding or granulating the dried material. Suitable methods of obtaining extracts of the above-mentioned plants or vegetables are known in the art. The plant or vegetable extracts are
 20 obtainable e.g. by extracting the fresh-cut or processed plants or vegetables or the respective roots, fruits or seeds thereof for example with water or with one or more food grade solvents or with a mixture of water and one or more food grade solvents. Preferably, the extracts and concentrates according to the present invention may be
 25 lipidic or aqueous. Since carotenoids are liposoluble, extraction with water will remove unwanted constituents which are water-soluble such as sugars, amino acids, soluble proteins, organic acids, for example.

30 If the carotenoid-containing material is obtained from microorganism, any microorganism that produces carotenoid may be used, in particular probiotic microorganism, such as lactic acid bacterium, for example. Also, the product of animal

origin may be from salmon, shrimps, for example or a liver extract or a milk fraction. The term "milk fraction" is understood to mean any part of the milk.

5 In a most preferred embodiment, the carotenoid-containing material is an oleoresin. Suitable methods of obtaining oleoresins from the above-mentioned plants or vegetables are well known in the art. For example, oleoresins are obtained by lipidic extraction using a solvent compatible with the food business, cosmetics or pharmaceuticals. Oleoresins prepared by conventional methods have a content in carotenoid of about 0.05% to 50% by weight. Their content of all-trans isomer of
10 carotenoids is usually higher than that of cis isomers, e.g. the ratio of cis-trans isomers of lycopene in a selected tomato oleoresin is about 7:93. Oleoresins are preferred starting material for obtaining the primary composition according to the present invention, because they contain other carotenoids or antioxidants such as Vitamine E, which also stabilize the composition. The activity and stability of the carotenoid
15 compound in the oleoresin is improved, in particular during the isomerisation process and the yield of the cis-lycopene in the primary composition is also increased.

The carotenoid-containing material preferably includes carotenes and xanthophylls, such as lycopene, carotene, zeaxanthine, astaxanthine, beta-cryptoxanthin,
20 capsanthine, canthaxanthine, lutein, phytofluene or phytoene, for example. Said carotenoid compounds have been subjected to a treatment to increase the cis-isomer fraction in the primary composition.

Accordingly, it is a further object of the invention, to provide a process for the
25 preparation of such a primary composition comprising the step of subjecting a carotenoid-containing material as described above, under conditions sufficient to increase its content in cis-isomers of carotenoid to an amount effective to increase the bioavailability and/or bioefficacy of the carotenoid compound in the primary composition.

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In a most preferred embodiment, the carotenoid-containing material which is in the form of an extract, a concentrate or an oleoresin, is subjected to a microwave irradiation or to other treatments including non-thermal treatments. Conditions of the microwave irradiation depend on the quantity and quality of the material. If an oleoresin is used, the power and time are adjusted so that the temperature of the microwave oven is of at least 100°C, preferably from 100 to 180°C and most preferably from 115 to 140°C. If an aqueous extract is used, a medium adapted to microwave irradiation may be used. The aim of the medium is to solubilize or disperse carotenoids. The losses can be minimised when the isomerisation is performed under nitrogen in the presence of antioxidants and in the absence of light. The isomerisation yield may also be improved by adding exogenous lipids in the medium.

The *cis:trans* isomer ratio in the primary composition may then be increased up to at least 20:80, preferably between 20:80 to 95:5; more preferably from 30:70 to 70:30.

The present invention thus provides a primary composition, in the form of a powder, liquid or gel, comprising a carotenoid compound which has a better bioavailability and/or bioefficacy than the compound alone. Also, the primary composition may be in the form of a highly water-dispersible composition, if the powder form is chosen. In this instance, the powder is dispersible in water at ambient temperature. The primary composition also provides carotenoids in a particularly highly soluble form in lipids and organic solvents, less prone to crystallization, and having a lower tendency to aggregate.

According to the invention the primary composition may be used either alone or in association with other active compounds such as vitamin C, vitamin E (tocopherols and tocotrienols), carotenoids (carotenes, lycopene, lutein, zeaxanthine, beta-cryptoxanthine, etc ..) ubiquinones (e.g. CoQ₁₀), catechins (e.g. epigallocatechin gallate), coffee extracts containing polyphenols and/or diterpenes (e.g. kawheol and cafestol), extracts of chicory, ginkgo biloba extracts, grape or grape seed extracts rich in

proanthocyanidins, spice extracts (e.g. rosemary), soy extracts containing isoflavones and related phytoestrogens and other sources of flavonoids with antioxidant activity, fatty acids, e.g. n-3 fatty acids, phytosterols, prebiotic fibers, probiotic microorganisms, taurine, resveratrol, aminoacids, selenium and precursors of glutathione, or proteins, such as whey proteins, for example.

The composition additionally comprises one or more of emulsifiers, stabilizers and other additives. Use is made of emulsifiers compatible in the food field, such as phospholipids, for example lecithin; polyoxyethylene sorbitan mono- or tristearate, monolaurate, monopalmitate, mono- or trioleate; a mono- or diglyceride. Use may also be made of any type of stabilizer that is known in the food business, in cosmetics or in pharmaceuticals. Use is made, as additives, of flavorings, colorants and any other additive known in the food business, in cosmetics or in pharmaceuticals. These emulsifiers, stabilizers and additives are added according to the final use of the primary composition.

According to a further object, the present invention relates to an oral composition comprising the primary composition described above in a foodstuff, in a food supplement, in a pet food product, in a cosmetic preparation or in a pharmaceutical preparation.

In a preferred embodiment, a food composition for human consumption is supplemented by the above primary composition. This composition may be a nutritional complete formula, a dairy product, a chilled or shelf stable beverage, a mineral water, a liquid drink, a soup, a dietary supplement, a meal replacement, a nutritional bar, a confectionery, a milk or a fermented milk product, a yogurt, a milk based powder, an enteral nutrition product, an infant formulae, an infant nutritional product, a cereal product or a fermented cereal based product, an ice-cream, a chocolate, coffee, a culinary product such as mayonnaise, tomato puree or salad dressings or a pet food.

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In this case, the powder is dissolved in the above-mentioned foods or drinks so as to have a daily intake of between about 0.001 and 50 mg of carotenoid contained in the primary composition, for example such as lycopene. A daily intake of the order of about 5 to 20 mg per day is preferably envisaged.

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The nutritional supplement for oral administration may be in capsules, gelatin capsules, soft capsules, tablets, sugar-coated tablets, pills, pastes or pastilles, gums, or drinkable solutions or emulsions, syrups or gels, with a dose of about 0.001 to 100% of the primary composition, which can then be taken directly with water or by any other known means. This supplement may also include a sweetener, a stabilizer, an additive, a flavoring or a colorant. A supplement for cosmetic purpose can additionally comprises a compound active with respect to the skin. Methods for preparing them are common knowledge.

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In another embodiment, a pharmaceutical compositions can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described herein under, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "a therapeutically effective dose". Amounts effective for this will depend on the severity of the disease and the weight and general state of the patient.

20

In prophylactic applications, compositions according to the invention are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be "a prophylactic effective dose". In this use, the precise amounts again depend on the patient's state of health and weight.

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The compounds of the invention are preferably administered with a pharmaceutical acceptable carrier, the nature of the carrier differing with the mode of administration, for example parenteral, intravenous, oral and topical (including ophthalmic) routes. The desired formulation can be made using a variety of excipients

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including , for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin cellulose, magnesium carbonate. This composition may be a tablet, a capsule, a pill, a solution, a suspension, a syrup, a dried oral supplement, a wet oral supplement, dry tube-feeding, wet tube-feeding etc..

5

Preferably, for humans the pharmaceutical composition according to the present invention comprises an amount of the primary composition as described above, for a daily administration, so that the carotenoid amount is from about 0.01 mg to 100 mg. When administered daily to pets, the carotenoid amount is from about 0.01 mg to 100

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It will be appreciated that the skilled person will, based on his own knowledge select the appropriate components and galenic form to target the active compound to the tissue of interest, e.g. the skin, colon, stomach, kidney or liver, taking into account the route of administration which may be by way of injection, topical application, intranasal administration, administration by implanted or transdermal sustained release systems, and the like.

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The invention also relates to a cosmetic composition comprising the primary composition described above. It may be formulated in lotions, shampoos, creams, sun-screens, after-sun creams, anti-ageing creams and/or ointments, for example. In this case, the content of primary composition is between 10.sup.-10 and 10%. The cosmetic composition preferably comprises between 10.sup.-8 and 5% of carotenoid compound. This composition which can be used topically additionally comprises a fat or an oil which can be used in cosmetics, for example those mentioned in the CTFA work, Cosmetic Ingredients Handbook, Washington. It is also possible to add other cosmetically active ingredients. The composition additionally comprises a structuring agent and an emulsifier. Other excipients, colorants, fragrances or opacifiers can also be added to the composition. It will be appreciated that the present cosmetic products will contain a mixture of different ingredients known to the skilled person, ensuring a fast

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penetration of the objective substance into the skin and preventing degradation thereof during storage.

5 It will be understood that the concept of the present invention may likewise be applied as an adjuvant therapy assisting in presently used medications. Since the compounds of the present invention may easily be administered together with food material special clinical food may be applied containing a high amount of the objective substances. It will be clear that on reading the present specification together with the appending claims the skilled person will envisage a variety of different alternatives to
10 the specific embodiments mentioned herein.

The present invention additionally relates to the use of the primary composition, or the oral composition or the cosmetic composition described above for the preparation of a composition intended to protect the tissues of the skin against ageing, in particular
15 for inhibiting damage to the skin and/or mucous membranes by inhibiting collagenases and enhancing the synthesis of collagen. In fact, the use of the primary composition as described above makes it possible to enhance the bioavailability of the carotenoid compound in the body and to slow down the ageing of the skin, for example. It may also be useful in the prevention or treatment of sensible, dry or reactive skins, or for
20 improving skin density or firmness, for ameliorating skin photoprotection, for preventing or treating cardiovascular diseases or disorders and cancers. They have also particular benefits on hair and coat of pet animals, such as an improved hair or coat density, fiber diameter, color, oilness, glossyness and a help to prevent hair or coat loss.

25 The effect of a food supplementation in the primary composition according to the present invention, on skin of humans or pets, can be measured by using conventional methods including minimal erythematous dose (MED), colorimetry, transepidermal water loss, DNA repair (e.g. p.53), measure of interleukines and proteoglycans production, or collagenase activity, barrier function or cell renewal or
30 ultrasonic echography.

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The following examples illustrate the invention in more detail without restricting the same thereto. All percentages are given by weight otherwise indicated.

Examples

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Example 1: Preparation of the primary composition

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25 g of lycopene oleoresin manufactured by LycoRed Natural Products Industries, Ltd. in Israel, are subjected to a microwave treatment of 150 sec. at 2.45 GHz at a temperature of about 105°C. The primary composition thus obtained is cooled down under nitrogen to prevent carotenoid degradation. The ratio of *cis:trans* isomer for lycopene is about 65:35 in the resulting composition.

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Example 2: Measure of isomerisation and degradation of lycopene in tomato oleoresin

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The isomerisation yield and degradation of a tomato oleoresin from LycoRed Natural Products Industries, Ltd. in Israel, which has been treated by conventional heating (oil bath) or microwave is measured.

25

For this, tomato oleoresin (Lycored 6%) in glass vials was irradiated in a microwave oven (2.45 GHz) for various periods of time up to 150 s. At the end of each exposure period the temperature was measured in the oleoresin sample. After cooling, the samples were dissolved in n-hexane (containing 200 ppm BHT) and analysed by HPLC for isomer formation. Additionally, tomato oleoresins in glass vials were heated in an oil bath to the same temperatures as those previously measured at the end of the microwave treatments. They were removed immediately from the oil bath, left for cooling and analysed in the same way as the microwave irradiated samples. The HPLC method for lycopene isomer determination was adapted from Schierle et al. (1997) Food Chem. 59, 459-65.

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The results are illustrated in Figures 1 and 2. Fig. 1 shows that predominantly 9- and 13-*cis* isomers of lycopene were generated by microwave irradiation, beside some other unidentified isomers and remaining all-*trans* lycopene. Fig.2 shows that lycopene isomerisation is stronger in the microwave treated oleoresin and the temperatures needed to generate *cis* isomers were lower compared to the conventionally heated samples.

In conclusion microwave is a very efficient method to generate geometrical isomers of lycopene.

10 Example 3: Cosmetic for oral administration

A composition in the form of a hard capsule has the following formulation:

Compound	mg per capsule
primary composition of example 1	250
Excipient for the coating core	
Microcrystalline cellulose	70
Encompress TM	60
Magnesium stearate	3
Anhydrous colloidal silica	1
Coating agent	
Gum-lac	5
Talc	61
Sucrose	250
Polyvidone	6
Titanium dioxide	0.3
Coloring agent	5

15 The composition can be administered to the individual in an amount of 2 to 3 capsules daily.

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Claims

1. A primary composition comprising at least one carotenoid-containing material, enriched in *cis*-isomers of the carotenoid compound.

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2. The primary composition according to claim 1, wherein the carotenoid-containing material is obtained, extracted or purified from a plant or vegetable material, microorganism, yeast or product of animal origin.

10

3. The primary composition according to claim 1 or 2, wherein the carotenoid-containing material is in the form of an extract, a concentrate or an oleoresin.

15

4. The primary composition according to one of claims 1 to 3, in which the *cis*-isomers of the carotenoid compound are in an amount effective to increase the bioavailability and/or bioefficacy of said carotenoid compound.

20

5. The primary composition according to one of claims 1 to 4, wherein the plant or vegetable material is tomatoes, carrots, peaches, apricots, oranges, melons, guavas, papayas, grapefruit, rosehips, soya, green tea, green coffee beans, spices, grapes or cocoa.

25

6. The primary composition according to one of claims 1 to 5, wherein the carotenoid compound is lycopene, carotenes, zeaxanthine, astaxanthine, β -cryptoxanthin, capsanthine, canthaxanthine, lutein, phytofluene or phytoene.

7. The primary composition according to one of claims 1 to 6, in which the *cis:trans* isomers ratio of the carotenoid compound is of at least 20:80.

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8. The primary composition according to one of claims 1 to 7, which is in liquid, gel or powder form.

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9. An oral composition comprising the primary composition according to one of claims 1 to 8, in a foodstuff, in a food supplement, in a pet food product, in a cosmetic preparation or in a pharmaceutical preparation.
- 5 10. The oral composition according to claim 9, wherein the foodstuff is chosen from the group consisting of a nutritional complete formula, a dairy product, a chilled or shelf stable beverage, a mineral water, a liquid drink, a soup, a dietary supplement, a meal replacement, a nutritional bar, a confectionery product, a milk or a fermented milk product, a yogurt, a milk based powder, an enteral nutrition product, an infant formulae,
10 an infant nutritional product, a cereal product or a fermented cereal based product, an ice-cream, a chocolate, coffee, a culinary product or a pet food product.
11. The oral composition according to claim 9 or 10, wherein the food supplement is provided in the form of capsules, gelatin capsules, soft capsules, tablets, sugar-coated
15 tablets, pills, pastes or pastilles, gums, drinkable solutions or emulsions, syrups or gels.
12. The oral composition according to claim 11, which further comprises at least one of a sweetener, a stabilizer, a flavoring and a colorant.
- 20 13. The oral composition according to claim 9, wherein the cosmetic preparation additionally comprises a compound active with respect to the skin.
14. The oral composition according to one of claims 9 to 13, wherein the content of the primary composition is between about 0.001 and 100%, and preferably between
25 about 10 and 50%.
15. A cosmetic composition comprising the primary composition according to one of claims 1 to 8.
- 30 16. The cosmetic composition according to claim 15, wherein the content of primary composition is between about 10.sup.-10% and 10%.

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17. A process for the preparation of a primary composition according to claim 1 to 8, which comprises subjecting the carotenoid-containing material to a treatment under conditions sufficient to increase its content in *cis*-isomers of the carotenoid compound.

5

18. The process according to claim 17, wherein the *cis*-isomer content is an amount effective to increase the bioavailability and bioefficacy of said carotenoid compound.

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19. The process according to claim 18, wherein the treatment is a thermal treatment, an electromagnetic irradiation or a radical reaction.

20. Use of a primary composition comprising at least one carotenoid-containing material enriched in *cis*-isomers, for the preparation of an oral, cosmetic or pharmaceutical composition for improving skin health.

15

21. The use according to claim 20, for protecting the tissues of the skin against ageing, in the prevention or treatment of sensible, dry or reactive skins, or for improving skin density or firmness, for increasing skin photoprotection.

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22. Use of a primary composition comprising at least one carotenoid-containing material enriched in *cis*-isomers, for the preparation of an oral, cosmetic or pharmaceutical composition for preventing or treating cardiovascular diseases or cancers.

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Abstract**Compositions containing *cis*-isomers of a carotenoid compound**

- 5 The present invention relates to a primary composition that includes at least one carotenoid-containing extract, concentrate or oleoresin enriched in *cis*-isomers of the carotenoid compound, and process of forming the same. It also relates to an oral composition that contains the primary composition in a foodstuff, in a food supplement, in a cosmetic preparation or in a pharmaceutical preparation.

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Figure 1: Typical HPLC chromatogram of lycopene isomers obtained by microwave irradiation of tomato oleoresin. Identified peaks are labelled.

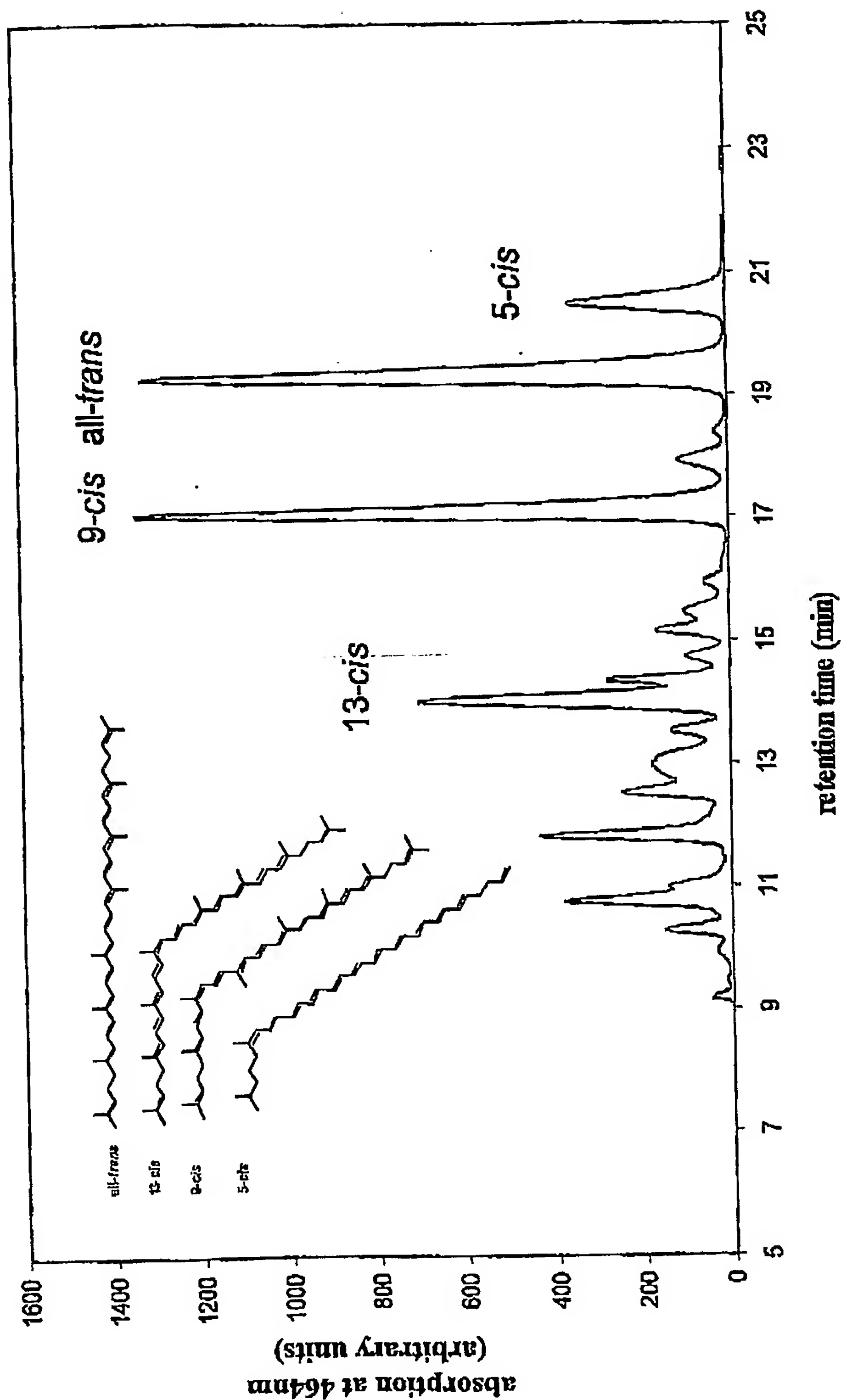


Figure 2: Isomerisation of lycopene in tomato oleoresin by microwave irradiation compared to conventional heating

